Date of Approval: May 8, 2015

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-438

KAVAULT

Avilamycin Type A Medicated Article

Swine

For the reduction in incidence and overall severity of diarrhea in the presence of pathogenic *Escherichia coli* in groups of weaned pigs.

Sponsored by:

Elanco Animal Health,
A Division of Eli Lilly & Co.

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I. GENERAL INFORMATION

A. File Number

NADA 141-438

B. Sponsor

Elanco Animal Health, A Division of Eli Lilly & Co.
Lilly Corporate Center
Indianapolis, IN 46285

Drug Labeler Code: 000986

C. Proprietary Name

KAVAULT

D. Established Name

Avilamycin Type A medicated article

E. Pharmacological Category

Antimicrobial

F. Dosage Form

Type A medicated article

G. Amount of Active Ingredient

90.7 g/lb (200 g/kg)

H. How Supplied

25 kg (55.12 lb) bag

I. Dispensing Status

VFD

J. Dosage Regimen

Feed at 73 grams avilamycin per ton of Type C medicated feed (80 ppm) as the sole ration for 21 consecutive days. The veterinarian may direct feeding for up to a total of 42 consecutive days, based on the clinical assessment.

K. Route of Administration

Oral

L. Species/Class

Swine/weaned pigs up to 14 weeks of age

M. Indication

For the reduction in incidence and overall severity of diarrhea in the presence of pathogenic *Escherichia coli* in groups of weaned pigs.

II. EFFECTIVENESS

A. Dosage Characterization:

Data collected from studies conducted in Mexico, Thailand, and Greece evaluated avilamycin effectiveness in newly weaned pigs following either oral *Escherichia coli* challenge in an induced post-weaning diarrhea model or natural infection following comingling without effort to minimize post-weaning stress. Pigs on study were assigned to one of five treatment groups: non-medicated feed (negative control), one of two positive control groups, or one of two avilamycin feed concentrations (40 or 80 ppm).

Approximately half of all animals with a natural colibacillosis infection due to comingling post-weaning had diarrhea by the tenth day post-weaning. All pigs were subsequently weighed, ear-tagged, then ranked according to body weight, sex, and severity of diarrhea (using a 0-3 scoring system) prior to random allocation to study pens.

Treatments were randomly assigned to study pens. Experimental ration administration was initiated on the day of pen allocation for a 28-day duration.

Avilamycin administered at 80 ppm reduced average daily diarrhea score, overall days with diarrhea, and mortality. Avilamycin administered at 40 ppm reduced mortality and trended toward reduction of diarrhea score.

A confirmatory study conducted in South Dakota evaluated the effectiveness of avilamycin in newly weaned pigs following oral *E. coli* challenge in an induced post-weaning diarrhea model. Pigs were assigned to one of four treatments: non-medicated feed (negative control), a positive control group, or one of two avilamycin feed concentrations (40 or 80 ppm).

Treatment feed administration was initiated on Study Day 0, and pigs were challenged with hemolytic *E. coli* on Study Days 4, 5, and 6. Treatment feeds were administered for the duration of the 32-day treatment phase. Each animal was observed daily from Study Day 0 through Study Day 32 for diarrhea, perianal inflammation, depression, and appetite scoring. Fecal samples were collected throughout the study for quantitative assessment of hemolytic *E. coli* shedding. Effectiveness was assessed based on relative differences between treatment groups for diarrhea scores to define prevalence, incidence, and severity of disease during the treatment phase. Pigs administered either avilamycin 40 ppm or avilamycin 80 ppm had statistically significant reductions in diarrhea incidence and severity relative to negative controls ($p < 0.05$). Diarrhea incidence in avilamycin 80 ppm and positive control treatments were significantly improved relative to avilamycin 40 ppm ($p < 0.05$).

Based on the results of these studies, an inclusion rate of 80 ppm avilamycin in the feed was chosen to evaluate in the clinical field effectiveness study.

B. Substantial Evidence:

1. Clinical Field Study

a. Title: "Clinical Study (GCP): Efficacy of Avilamycin Administered in Feed for Reduction in Incidence and Severity of Nursery Pig Colibacillosis." Study Numbers T4EUS100011, T4EUS100012, T4EUS110002, and T4EUS110003 (March 2011 to May 2011).

b. Investigators and Study Locations:

(1) T4EUS100011
Lyle Kesl, DVM, Ph.D.
Veterinary Resources, Inc. (VRI) Twedt Facility
Williams, IA

(2) T4EUS100012
Ryan Saltzman, DVM
VRI Schwartz Facility
Story City, IA

(3) T4EUS110002
Kelly Lechtenberg, DVM, Ph.D.
Central States Research Centre, Inc.
Oakland, NE

(4) T4EUS110003
Terry TerHune, DVM, Ph.D.
HMS Veterinary Development, Inc.
Tulare, CA

c. Study Design:

(1) Objective: To demonstrate effectiveness of avilamycin Type A medicated article administered in feed for reduction in incidence and overall severity of diarrhea in the presence of pathogenic *Escherichia coli* in groups of newly weaned pigs during the nursery production phase.

(2) Study Animals: At each site, a total of 200 newly-weaned, crossbred female and castrated male pigs were enrolled in the study. Pigs were approximately 3 to 4 weeks of age at receipt, and weighed approximately 5 to 10 kg. Pigs were sourced from commercial U.S. swine production facilities and were uniquely identified with bilateral, identical ear tags. The pigs were housed in indoor, concrete pens with either solid or slatted flooring representative of industry standards, and mechanical and/or curtain-sided ventilation. A commercial-type feed ration appropriate for the pigs' age and stage of production was provided *ad libitum* throughout the study. Non-medicated water was provided *ad libitum*. Study animals were enrolled and randomly allocated to pen and treatment if they met the following criteria:

- Good health based on physical examination conducted by study investigator on Study Day 0

- Study Day 0 Clinical Observation Scores within normal parameters (see also Tables 1, 2, and 3 below):

  - Diarrhea Score ≤ 1, AND
  - Depression Score = 0, AND
  - Gauntness Score = 0

- No history of diarrheal disease or vaccination for *E. coli*

- History of exposure to one or more risk factors associated with post-weaning colibacillosis (such as weaning, transport, relocation, commingling, or diet change) at the time of enrollment

(3) Treatment Groups: The test article was avilamycin Type A medicated article (100 g/kg), administered as a pelleted Type C medicated feed at an inclusion rate of 80 ppm avilamycin. Non-medicated feed was the control article. At each site, 10 pens (100 pigs) were assigned to each of the two treatments. Across the study, 400 pigs were enrolled in the avilamycin group, and 400 pigs were enrolled in the control group.

(4) Drug Administration: Following allocation of animals to pens on Study Day 0, test and control feeds were issued to each pen for 21 consecutive days (Study Days 0 to 21, treatment phase). Feed was issued on an "as needed" basis to ensure continual access to feed, except during brief periods such as when animals or feed were weighed.

(5) Measurements and Observations: After enrollment, pigs were evaluated for diarrhea, depression, and gauntness scores at the time of physical examination on Study Day 0 and daily on Study Days 1 to 21. The scoring criteria and scales are presented in Tables 1, 2, and 3. Rectal swab samples were collected from each animal on Study Days 1 to 7 for microbiologic analysis to isolate beta-hemolytic *E. coli*. During the post-treatment phase on Study Days 22 to 28, remaining study animals were observed for abnormal health conditions and were fed a non-medicated ration *ad libitum*.

Table 1. Diarrhea scoring scale

Clinical Score	Clinical Sign
0	Normal feces; no diarrhea present
1	Semi-solid (cow-pie consistency)
2	Loose with some solid material (oatmeal consistency)
3	Watery feces with little or no solid material

Table 2. Depression scoring scale

Clinical Score	Clinical Sign
0	Bright, alert, and responsive.
1	May stand isolated but will quickly respond to stimulation
2	May stand isolated with head down and possible signs of muscle weakness; delayed response to stimulation
3	Severely depressed; recumbent and reluctant to rise

Table 3. Gauntness scoring scale

Clinical Score	Clinical Sign
0	Normal abdominal fill; flank is full and round
1	Decreased gut fill; flank is flat
2	Severely gaunt; flank is hollow

d. Statistical Analysis: The individual animal was the observational unit, and pen was the experimental unit. The primary variables for determining effectiveness were diarrhea incidence and mean diarrhea severity score. All statistical tests for effectiveness variables were two-tailed tests performed at the 5% significance level.

Diarrhea incidence was calculated as the percentage of animals in the effectiveness evaluable population (number animals enrolled minus non-colibacillosis removals) with diarrhea score ≥ 2 on one or more study days during the treatment phase. For analysis of diarrhea incidence, a generalized linear mixed model (Proc Glimmix, SAS v9.1.3 or newer) was fit to pen-based counts with Treatment as the fixed effect and Site, Treatment*Site, and Block (Site) as random effects assuming a binomial distribution and a logit link.

Mean diarrhea severity score for each treatment group was calculated as the sum of diarrhea scores for each treatment group divided by the sum of days in treatment of all the animals not removed for non-colibacillosis reasons. The mean diarrhea severity score was transformed via arsin ((DIAR_MEAN/3)*0.5). The transformed mean diarrhea severity scores were analyzed using a linear mixed model (Proc Mixed, SAS v9.1.3 or newer) with Treatment as the fixed effect and Site, Treatment*Site, and Block (Site) as random effects.

e. Results: Thirty-seven pigs were removed from the study for non-colibacillosis illness or protocol deviations and were excluded from the effectiveness analysis. A statistically significantly lower ($p = 0.0075$) incidence of diarrhea (based on least squares [LS] means) was detected for the treated group (62.44%) compared to the control group (90.78%). A statistically significantly lower ($p = 0.0065$) mean diarrhea severity score (based on back-transformed LS means) was detected for the treated group (0.43) compared to the control group (0.87). Beta-hemolytic *E. coli* were isolated from rectal swab samples collected from 69 to 98% of study animals at each site.

f. Adverse Reactions: There were no test article-related adverse reactions in this study.

g. Conclusions: This study demonstrates that KAVAULT (avilamycin Type A medicated article) is effective for the reduction in incidence and overall severity of diarrhea in the presence of pathogenic *E. coli* in groups of weaned pigs, when administered as the sole ration in complete feed at 80 ppm for 21 consecutive days.

III. TARGET ANIMAL SAFETY

A. Margin of Safety Study

1. Title: "Non-Clinical Laboratory Study (GLP): Target Animal Safety Evaluation of Avilamycin in Weaned Piglets." Study Number MCL 1055, March 2011 to March 2012.

2. Study Director: Teresa Schieber, DVM. Midwest Veterinary Services, Inc., Oakland, NE

3. Study Design:

   a. Objective: The objective was to determine the margin of safety associated with feeding avilamycin at 0X (negative control), 1X, 3X, and 5X the labeled dose (80 ppm) to newly weaned pigs (21±4 days of age at the start of acclimation phase) for 91 days (13 weeks).

   b. Study Animals: The test animals were healthy, intact female and male commercial crossbred pigs, which, at the start of treatment, were between 32 and 39 days of age and weighed between 8.6 to 13.1 kg. The animals were acclimated for 14 days prior to the first day of treatment.

   c. Treatment Groups: The study consisted of six blocks with eight pens per block in a randomized complete block design. A total of 48 animals were enrolled with one animal per pen. Each block included four pens in close proximity for each of the two genders (male and female). For each gender within a block, each of the four treatment groups (0X, 1X, 3X, and 5X) was randomly assigned to one piglet within each gendered section for each block. The animals were assigned to treatment and control groups as shown in Table 4. The animals were further randomly subdivided into two subgroups, A and B, each consisting of exactly half of the blocks. Study Day 0 was the same for both subgroups, with certain study procedures staggered for each subgroup by one calendar day. This was done to ensure sufficient time for processing the animals for data collection throughout the study.

Table 4. Summary of Treatment Groups

Group	Treatment Regimen	Number of Animals
Negative Control	0 ppm avilamycin	12 (6 males and 6 females)
1X	80 ppm avilamycin	12 (6 males and 6 females)
3X	240 ppm avilamycin	12 (6 males and 6 females)
5X	400 ppm avilamycin	12 (6 males and 6 females)

d. Drug Administration: The test article was avilamycin Type A medicated article (100 g/kg), administered orally as a Type C medicated feed. The control article was unmedicated feed.

Treatments were administered orally in pelleted swine feed appropriate for the animals' age and stage of production. The feed contained either 0 ppm avilamycin (negative control), 80 ppm avilamycin, 240 ppm avilamycin, or 400 ppm avilamycin, and was provided *ad libitum* for 91 days.

e. Measurements and Observations: The following parameters were measured and/or observed during the study period: clinical observations (including physical examination, general health observations, feed and water consumption, and body weight), fecal analysis, clinical pathology, urinalysis, and post-mortem (gross and microscopic) examination.

General health observations were made once daily from Study Day -14 until Study Day -1. Beginning on Study Day 0 (the start of treatment), general health observations were made twice daily, at least 6 hours apart, up to the day of necropsy.

For all pigs, physical examinations were performed on the pigs two days before treatment began (Study Day -2). For Subgroup A, physical examinations were performed on Study Days 21, 42, 63, and 90. For Subgroup B, physical examinations were performed on Study Days 22, 43, 64, and 91.

Feed was issued to each pen as necessary to provide free-choice feed availability. Feed weighback was conducted weekly, at each diet change, and as necessary if feed was out of condition.

Water was available to each pen to provide free-choice consumption. Water consumption was estimated based on weekly water meter readings.

Fecal sampling was performed in connection with blood sampling on Study Days -1 (baseline), 21, 42, 63, and 90 for Subgroup A and Study Days 0 (baseline), 22, 43, 64 and 91 for Subgroup B. The feces were visually assessed within fecal flotation containers for color and consistency, and for the presence of frank blood and parasites. The feces were assessed

microscopically for the presence or absence of parasites. The feces were assessed for the presence of occult blood by an occult blood test card.

Clinical pathology, consisting of hematology and clinical chemistry, was assessed on the following days: for all pigs on Study Day -6 (pretreatment clinical evaluation prior to randomization and enrollment); for Subgroup A on Study Days -1 (baseline), 21, 42, 63, and 90; and for Subgroup B on Study Days 0 (baseline), 22, 43, 64, and 91.

Animals were weighed upon arrival at the test facility (Study Day -14); at the veterinary physical examination (Study Day -2); at Study Days 21, 42, 63, and 90 for Subgroup A; at Study Days 22, 43, 64, 91, and 92 (Day 92 was a noted deviation that had no impact on the study results) for Subgroup B; and prior to each feed change (Study Days 14 and 35 for Subgroup A, and Study Days 15 and 36 for Subgroup B).

All study animals were euthanized over two days (Study Days 91 and 92 for Subgroups A and B, respectively). A masked veterinary pathologist performed the gross necropsy on each animal. Urine specimens for urinalysis were collected via cystocentesis at necropsy. Organ weights were obtained for the brain, heart, liver, and kidneys. Heart, liver, and kidney weights were then compared to brain weights. Samples from normal tissues and from gross lesions were collected for histopathology.

4. Statistical Analysis:

For the randomized complete block design that was conducted, two factors, Gender and Treatment, were investigated for all variables. For some variables, a third factor, Time, was also investigated. For those variables which included Time as a factor, a linear mixed model analysis, Proc Mixed SAS®, was conducted with fixed effects Treatment, Gender, Time, two and three factor interactions, and a covariate in some cases with a random effect, Block. The two and three factor interactions were initially tested ($p < 0.05$) to determine if separate analyses should be conducted for Gender and/or Time. Pair-wise comparisons were conducted comparing the non-zero levels of Treatment to control at a significance level of 0.10. Least squares (LS) means and standard errors as well as arithmetic means and standard deviations were calculated for each Treatment level (0, 80, 240, and 400 ppm avilamycin).

For those variables which did not have Time as a factor, a linear mixed model, Proc Mixed SAS, was conducted with fixed effects Treatment, Gender, and Treatment x Gender interaction and a random effect, Block. Initially, the Gender x Treatment interaction was tested and if significant ($p < 0.05$), separate Gender analyses were conducted with fixed effect Treatment and random effect, Block. Pair-wise comparisons were conducted comparing the non-zero levels of Treatment to control at a significance level of 0.10. LS means and standard errors as well as arithmetic means and standard deviations were calculated for each Treatment level (0, 80, 240, and 400 ppm avilamycin).

For variables associated with adverse events, a relatively small number of observations were made. Therefore, Fisher's exact test was conducted with a significance level of 0.10.

5. Results:

   a. Clinical Observations: Clinical observations included physical examination, pen observations, feed and water consumption, and body weight. Near the end of the study, Animal #108 lacerated its right rear toe, which caused severe lameness. Because of the laceration and severe lameness, Animal#108 was euthanized for humane reasons and necropsied one day prior to scheduled necropsy. No clinical observation findings were considered to be drug-related.

   b. Fecal Analysis: Fecal consistency was normal for all animals at all observation points, and frank blood was not observed for any animals at any time. Occult blood was detected in fecal samples prior to test article administration and continued to be observed across all treatment groups throughout the duration of the study, but was not considered to be clinically relevant. The positive occult blood results were not considered attributable to test article administration.

   c. Hematology: Statistically significant differences between various groups were detected in the following hematology parameters: mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), percent eosinophils, absolute monocyte count, platelet count, absolute white blood cell (WBC) count, absolute basophil count, hematocrit, and absolute and relative reticulocyte counts. These findings were not considered clinically relevant because the differences were small, did not consistently persist over time, did not indicate a dose- or duration-dependent trend, were not correlated to gross or clinical findings, and values remained within the normal reference range.

   d. Blood Coagulation: The activated partial thromboplastin time (APTT) in females was statistically significantly shorter in the 3X and 5X treatment groups than in the control group ($p = 0.0628$ and $p = 0.0041$, respectively). These findings were not considered clinically relevant because the differences were small, the values for treated animals were within normal reference ranges, and the observations were not correlated to gross or clinical findings.

   e. Serum Chemistry: Statistically significant differences between the control group and one or more treated groups were observed for glucose, potassium, sodium, alanine aminotransferase (ALT), and total protein. No trends were found for any of the serum chemistry parameters, and the differences between the treated and control groups were too small to be of clinical relevance. Therefore, these findings were considered incidental and not related to treatment.

   f. Urinalysis: There were no statistically significant or clinically relevant differences in the urinalysis results between the treatment and control groups.

   g. Post-mortem (gross and microscopic) Examination: Post-mortem gross and microscopic changes were distributed across all groups, were sporadic in occurrence, and were attributed to spontaneous changes commonly observed in pigs of this age or to euthanasia. A statistically significant increase in heart weight was observed between the 3X treatment group

compared to the control group (LS Means 369.08 g vs 336.62 g, respectively; p = 0.0067). A statistically significant decrease in liver weights relative to brain weights was observed between the female 3X treatment group and the female control group (LS Means 15.45 vs 17.43, respectively; p = 0.0734). No other statistically significant differences were detected in organ weights. No dose-related trends were observed with respect to the absolute increase in heart weight and the decrease in liver weight relative to brain weight, and the observations were not correlated to any clinical, gross necropsy, or microscopic observations. Therefore, it was concluded that there were no post-mortem findings attributable to avilamycin.

6. Conclusions: This study demonstrated that KAVAULT (avilamycin Type A medicated article) is safe for use in swine when administered as a Type C medicated feed containing 80 ppm avilamycin for 42 consecutive days.

IV. HUMAN FOOD SAFETY

A. Microbial Food Safety (Antimicrobial Resistance):

The Agency evaluated microbial food safety information and data for avilamycin, *"For the reduction in incidence and overall severity of diarrhea in the presence of pathogenic* Escherichia coli *in groups of weaned pigs."* Avilamycin is proposed for use in pigs that are at risk of developing, but not yet showing clinical signs of, diarrhea in the presence of pathogenic *Escherichia coli,* because avilamycin has not been demonstrated to be effective in pigs showing actual clinical signs of diarrhea. In addition, avilamycin has no microbiological (-cidal or –static) effects against *E. coli*.

The microbial food safety assessment submitted for Agency review included a *release assessment* to describe the probability that avilamycin and its use at 80 ppm for 42 days in a Type C medicated feed will result in the emergence of resistant bacteria or resistance determinants in treated swine under proposed conditions of use; an *exposure assessment* to describe the likelihood of human exposure to resistant bacteria or resistance determinants through consumption of edible products from avilamycin-treated swine; and a *consequence assessment* to describe potential human health consequences arising from exposure to defined resistant bacteria or resistance determinants by considering the human medical importance of orthosomycins used in the treatment of human infectious diseases.

The risk assessment included information on avilamycin, specifically its spectrum of antibacterial activity, mechanism(s) of avilamycin resistance and impact on the development or selection of antimicrobial resistance in gram positive foodborne pathogens of concern (*Enterococcus, methicillin-resistant Staphylococcus aureus*, and *Clostridium difficile*) as a result of the use of avilamycin in swine. Gram negative organisms of human health importance such as *Campylobacter, E. coli,* and *Salmonella* were not considered a hazard in this risk assessment as they are intrinsically resistant to avilamycin. This will be the first approved use of avilamycin in veterinary medicine in the United States. In addition, there are no analogs used in human clinical medicine, and as such, there was no data to fully assess the potential impact on human health from the subsequent emergence of antimicrobial resistance as a result of the use of avilamycin in swine.

The Agency concludes that use of avilamycin in swine will not result in a significant risk to the development of avilamycin resistance in foodborne *Enterococcus* originating from treated swine, based upon evaluation of the information submitted by the firm, consideration of the spectrum of activity of avilamycin, the potential of avilamycin to select for the subsequent emergence of antimicrobial resistance in treated swine, the prevalence of *Enterococcus* in swine-derived food products, and taking into considerations the following labeled conditions of use for avilamycin in swine feed:

- Avilamycin is indicated, "*For the reduction in incidence and overall severity of diarrhea in the presence of pathogenic* Escherichia coli *in groups of weaned pigs*",
- Avilamycin labeling will state, "*Feed at 73 grams avilamycin per ton of Type C medicated feed (80 ppm) as the sole ration for 21 consecutive days. The veterinarian may direct feeding for up to a total of 42 consecutive days, based on the clinical assessment.*", and "*Do not administer medicated feed containing avilamycin to pigs for more than a lifetime total of 42 days.*",
- Avilamycin will be available as a veterinary feed directive (VFD) and, therefore, will be administered under veterinary oversight,
- VFDs for avilamycin shall not be refilled,
- Avilamycin will be administered to weaned pigs and will not be used in pigs 14 weeks of age or older, thereby allowing an "inherent" withdrawal period of over 70 days (based on current industry practices).

The overall risk estimation associated with the use of avilamycin in feed for swine under the proposed conditions of use is medium, based on individual rankings of medium for the *release assessment*, high for the *exposure assessment*, and low for the *consequence assessment*. The latter ranking of low for the *consequence assessment* is based on the current lack of use of avilamycin or analogs in human medicine. The use of avilamycin in feed for swine, with the risk mitigations listed above, should assure the safe use of avilamycin, and in a manner that would mitigate resistance emergence or selection associated with any adverse impact on human health.

B. Impact of Residues on Human Intestinal Flora

1. Determination of the need for establishing a microbiological ADI (mADI)

A step-by-step approach, supported with study data, was followed to determine whether there is a concern for effects of avilamycin residues on human intestinal flora.

a. Step 1: Are residues of avilamycin and/or its metabolites microbiologically active against representative human intestinal bacteria?

Yes, avilamycin is active against representative human intestinal bacteria. This conclusion was supported by an *in vitro* susceptibility study performed by the firm, which is described below.

Study title: "Antibacterial activity of avilamycin: determination of minimum inhibitory concentrations (MIC) against 100 bacterial strains representing the normal human gut flora."

Study #: DWS/010/10

Report Date: June 4, 2010
Study Director: Dr. Andrew Pridmore
Study Location: Don Whitley Scientific Limited, West Yorkshire, United Kingdom

Summary: The minimum inhibitory concentration (MIC) of avilamycin against 100 bacterial strains

Ten isolates from each of 10 bacterial groups representing normal human intestinal microbiota were studied. The testing system was the agar dilution method as described and recommended by the Clinical and Laboratory Standards Institute (CLSI). For each isolate used in the MIC testing, the standardized inoculum was enumerated according to the recommended method for anaerobic and facultative anaerobic bacteria.

Table 5. **Minimum inhibitory concentration (µg/mL) of avilamycin** against representative bacterial groups from healthy human subjects

Bacterial group	MIC_{50}	MIC_{90}	MIC range
Bacteroides fragilis group	4	32	2 to > 128
Bacteroides, other species	32	64	16 to > 128
Bifidobacterium spp.	32	32	32 to > 128
Clostridium spp.	2	8	1 to 8
Enterococcus spp.	4	4	All 4
Escherichia coli	> 128	> 128	All > 128
Eubacterium spp.	2	16	0.5 to > 128
Fusobacterium spp.	8	8	1 to 8
Lactobacillus spp.	> 128	> 128	8 to > 128
Peptostreptococcus spp.	1	8	0.125 to 8
All isolates (# of 100)	8	> 128	0.125 to > 128

Results and Conclusions: As shown in Table 5, avilamycin had no measurable activity against *Escherichia coli* as judged by MIC_{50} and MIC_{90} at > 128 µg/ml, respectively. *In vitro* activity against other strains was variable, both within and among bacterial groups. Avilamycin also had relatively poor activity against *Lactobacillus* and *Bifidobacterium*.

The most susceptible species were *Peptostreptococcus*, *Eubacterium*, and *Clostridium*. The lowest MIC was against *Peptostreptococcus*, with MIC_{50} of 1 µg/mL.

b. Step 2: Do residues of avilamycin and its metabolites enter the human colon?

Yes, at least some of the avilamycin and its metabolites entering the colon are expected to be biologically active, even though the firm's study showed that there will be some reduction of activity due to binding of avilamycin and its metabolites to fecal materials.

c. Step 3: Do residues of avilamycin and its metabolites entering the colon remain microbiologically active?

Yes, the amount of avilamycin and its metabolites entering the colon is presumably biologically active. The firm conducted a study and found that the activity of avilamycin residues entering the colon will be reduced due to binding to fecal materials. The study is summarized below.

Study title: "Effect of fecal binding on antibacterial activity of avilamycin."

Study #: DWS/027/04
Report Date: October 22, 2004
Study Director: Dr. Andrew Pridmore
Study Location: Don Whitley Scientific Limited, West Yorkshire, United Kingdom

Summary: Binding of avilamycin to human feces was quantitatively measured by incubation of various concentration of avilamycin with increasing concentrations of sterile feces at 0, 10, 25, and 50% (w/v) in Mueller Hilton broth. Following incubation of each drug-fecal combination for a period of between 0 and 12 hours, microbiological concentration of avilamycin in the liquid portion of each mixture was assayed with a testing strain of *Enterococcus faecalis* with an MIC in the range of 2 to 4 µg/mL. The liquid portion was obtained by centrifugation at a low speed. Antibacterial activity in each inoculated preparation was assessed after 24-hour and 48-hour incubation by the presence or absence of bacterial growth, which provides an indication of the unbound concentration of avilamycin in each preparation. The calculation of fecal binding is estimated as the percent decrease in antibacterial activity according to the following formula:

$$\% \text{ bound} = (\text{MIC in feces} - \text{MIC in broth}) / \text{MIC in feces} \times 100$$

Results and Conclusions: Binding of avilamycin to feces in 10% and 25% fecal suspensions was time-dependent, with average binding of 60% and 95% at 6 and 12 hours of incubation, respectively. However, binding was rapid and irreversible when incubating in a 50% fecal suspension, with > 95% of fecal binding observed.

d. Step 4: Is there any scientific justification to eliminate testing of either colonization barrier disruption or resistance development endpoints?

The amount of avilamycin residues under the proposed conditions of use in swine will result in low concentrations of avilamycin and its metabolites in the colon. These low concentrations, when compared with the *in vitro* activity data in Step 1, are expected to have negligible effects on human intestinal flora.

The justification of no concern of avilamycin residues on human intestinal flora is summarized below.

Based on the residue depletion study (study ABC-0360 – described in section F–Residue Chemistry) that measured avilamycin residues in various

swine tissues at zero withdrawal time following feeding of avilamycin to swine at 60 mg/kg body weight for 14 days, a conservative, worst case analysis concluded that the avilamycin residue concentration in the colon will be 0.0007 µg/gram. Using the most susceptible bacterial group, *i.e.*, *Peptostreptococcus* spp. with an MIC_{50} of 1.0, and without considering the loss of activity due to fecal binding, the worst case concentration of avilamycin residues in the human colon would be > 1400-fold lower than the MIC_{50}. Therefore, the amount of avilamycin residues in the colon is too low to have an effect on human intestinal flora and there is no need to further evaluate either the barrier disruption or resistance development endpoint of concern.

2. Determination of the final mADI

As concluded from the assessments in the steps above, there is no need to determine a mADI under the proposed application.

Decision Statement: Under the proposed conditions of use, the amount of avilamycin residues reaching the human colon and remaining microbiologically active is negligible, and is not expected to have adverse effects on human intestinal flora.

C. Toxicology:

1. Summary of Toxicology Studies

a. Chronic Oral Toxicity Study in Non-Rodents

(1) Study Title: "A Chronic Toxicity Study of Avilamycin (Compound 48740, EL-750) Administered Orally to Dogs for Six Months"

(2) Study Number: D03782

(3) Report Number: 250-01549

(4) Report Date: November, 1983

(5) Study Director: Gail D. Williams, D.V.M., Ph.D.

(6) Performing Laboratory (in-life): Lilly Research Laboratories, Greenfield, IN, USA

(7) Experimental Design: The purpose of this study was to evaluate the effects of avilamycin on beagle dogs following oral administration of avilamycin for 6 months. Mycelium avilamycin (dried fermentation product) with avilamycin activity of 17.8% was administered orally to 32 dogs (4/sex/group) by gelatin capsule at 0, 20, 200, or 1000 mg/kg body weight (bw)/day (equivalent to 0, 3.56, 35.6, and 178 mg/kg bw/day, respectively). Empty gelatin capsules served as controls. Animals were observed daily for clinical signs. Body weight and food consumption were recorded, and eye examinations were performed. Blood and urine samples were collected from each group at pre-treatment, 2 weeks, 1 month, 2 months, 4 months, and at the end of the study. At necropsy, absolute organ weights were measured, and organ weight to brain and organ weight to body weight ratios were

calculated. Tissue pathology and bone marrow smear examinations were performed.

(8) Results and Conclusion: No treatment-related mortality, clinical signs of toxicity, hematology, urinalysis, organ weights, and pathology were observed. Blood chemistry parameters were not different from the control except for serum alanine transaminase (ALT), which was slightly increased on day 14 in males (1/4) and females (2/4) in the high dose group, and recovered thereafter. This finding was not associated with any other clinical chemistry or hepatic parameters, and was considered of no toxicological significance. A No-Observed-Effect Level (NOEL)/No-Observed-Adverse-Effect Level (NOAEL) of 1000 mg/kg bw/day for the dried fermentation product (equivalent to 178 mg/kg bw/day of avilamycin activity) was established in this study based on the absence of any toxicologically or biologically significant treatment-related effects.

b. Oral Developmental Toxicity Study in Rodents

(1) Study Title: "Embryo-fetal Developmental Study in Female CD Rats Given Granular Avilamycin Daily by Gavage"

(2) Study Number: R00228

(3) Report Number: R00228

(4) Report Date: November 3, 2004

(5) Study Director: Nancy J. Lawler

(6) Performing Laboratory (in-life): Eli Lilly Co., Greenfield, IN, USA

(7) Experimental Design: This study was conducted to determine the potential adverse effect of granular avilamycin on pregnant female rats, embryos, and fetal growth and development following daily treatment of dams from gestation days (GDs) 6 to 19. Granular form of avilamycin (26.4% avilamycin activity) in a water suspension was administered to time-mated pregnant rats (Crl:CD (SD) IGS BR), 25/group, by oral gavage at 500, 1000, and 2000 mg/kg bw/day (equivalent to 132, 264, and 528 mg avilamycin activity/kg bw/day) from GDs 6 to 19. The vehicle control group received water at 10 mL/kg bw/day. The highest dose, 2000 mg/kg bw/day, was chosen based on formulation feasibility. This study generally followed VICH Guideline (GL) 32 (2003). All dams were observed daily for clinical signs during the treatment period. The dams were sacrificed at GD 20 followed by Cesarean section. Maternal parameters collected included uterine weight, the number of corpora lutea per ovary; the number of implantation sites; live and dead fetuses; and the number of late and early resorptions. Live fetuses were weighed and examined for sex and external anomalies. Approximately one-half of the fetuses were examined for visceral anomalies, and the remaining fetuses were eviscerated and processed for skeletal examinations.

(8) Results and Conclusion: There were no treatment effects on body weight, food consumption, survival rates, clinical signs, and findings of

gross examination in the dams across treatment groups. One dam in the 1000 mg/kg bw/day group died on GD 18, with the cause of death pathologically determined to be a gavage error. Red urine (presumably bloody urine) was observed in several dams in three avilamycin treated groups (4/25 dams in both groups of 500 and 1000 mg/kg bw/day, 1/25 dam in the group of 2000 mg/kg bw/day). The nature of this finding was not conclusive due to 1) the incidence was low, 2) it was transient (only seen once in each affected animal), 3) the response was not dose-dependent, 4) a pilot study of similar treatments did not report this finding, and 5) the same observation was noted in control animals in an unrelated study in the same laboratory at about the same time. No treatment effect was observed at any doses in maternal reproductive parameters examined. Those parameters included corpora lutea, implantations, pre-implantation losses, resorptions, live fetuses, and post-implantation losses. No treatment-related effects were observed at any doses in fetal parameters examined, including fetal weight (both total and per sex per litter), sex ratio, fetal runts, normal fetuses, malformed fetuses, and fetuses with deviations and variations. Kidney cavitation and ureter dilation were seen in all groups, including the control. The incidences were similar among the dose groups and were within the historical controls of the study site. A NOEL/NOAEL was established at 2000 mg/kg bw/day for granular avilamycin (equivalent to 528 mg/kg bw/day of avilamycin activity) in this study, the highest dose tested.

c. Oral Developmental Toxicity Study in Non-Rodents

(1) Study Title: "A Teratology Study of Avilamycin (Compound 48740, EL-750) Administered Orally to Dutch Belted Rabbits"

(2) Study Number: B03482

(3) Reference Date: 1983

(4) Study Authors: Williams, G.D. & Hagopian, G.S.

(5) Performing Laboratory (in-life): Eli Lilly Co., Greenfield, IN, USA

(6) Experimental Design: In this study, fifteen pregnant Dutch belted rabbits per treatment group received daily gavage doses of 0, 250, 716, and 2000 mg/kg bw/day of dried fermentation product of avilamycin (17.8% avilamycin activity) from GDs 6 to 18. These doses were equivalent to 0, 44, 127, and 356 mg avilamycin activity/kg bw/day. The rabbits were sacrificed on GD 28 for morphological evaluation of the fetuses.

(7) Results and Conclusion: No skeletal or organ dysmorphogenesis was seen in the fetuses. Sternal anomalies and supernumerary ribs were observed in both control and treated animals, and were not considered treatment-related effects. It was concluded that avilamycin was not a teratogen in rabbits, and the NOEL/NOAEL for this study was 2000 mg/kg bw/day for the dried fermentation product (equivalent to 356 mg avilamycin activity/kg bw/day), the highest dose tested.

d. Three-Generation Oral Reproductive Toxicity Study in Rats

(1) Study Title: "Effect of CGA-59327 (avilamycin) on Reproductive Function of Multiple Generations in the Rat"

(2) Study Number: CBG/188/80780

(3) Report Date: April 13, 1981

(4) Study Authors: Palmer, A.K., Bottomley, A.M., Leeming, N.M., Clark, R., Offer, J.M. & Gibson, W.A.

(5) Performing Laboratory (in-life): Huntingdon Research Centre, Huntingdon, Cambridgeshire, England

(6) Experimental Design: In this three-generation reproductive toxicity study in Sprague-Dawley rats, dietary admixtures containing avilamycin as mycelial cake were administered at 0, 30, 300, and 3000 ppm (equivalent to 0, 1.5, 15, and 150 mg/kg bw/day based on 7% avilamycin activity) to females. An additional group (positive control) of 3000 ppm was given pure avilamycin (100% avilamycin activity). The study design allowed the production of three generations of rats from the initial animals (F0 rats, 25 per sex per dose). Rats in each generation were maintained on their treatment diets for at least 90 days prior to and during mating, and through gestation and lactation. For each generation, subsets of animals (dams or offspring, as appropriate) at each dose level were selected for 1) evaluation of absolute organ weight and organ weight changes of adult rats at the end of 90 day treatment, 2) morphological evaluation of fetuses at GD 20, and 3) growth and morphological evaluation of pups at postnatal day 21. Sperm parameters (sperm count, motility, and morphology) were not examined in this study.

(7) Results and Conclusion: The reproductive parameters examined included pregnancy rate, mating performance, gestation length, litter size, litter and mean pup weights, fetal mortality, pre- and post-implantation loss, embryotoxicity, and organ and skeletal dysmorphogenesis. There was no mortality or clinical signs that were attributable to avilamycin treatment. There were no consistent treatment-related changes in food and water consumptions, body weight, and reproductive parameters across the dose groups. Fetal developmental evaluation revealed that fetuses from F0 and F1 dams on GD 20 showed extra 14^{th} ribs, with an incidence ratio ranging from 5.0 to 11.4% in avilamycin-treated groups (8.3–8.8% for 1.5 mg/kg bw/day, 7.4–8.7% for 15 mg/kg bw/day, 5.0–11.4% for 150 mg/kg bw/day for mycelial cake, and 7.7% for 150 mg/kg bw/day for pure form) in contrast to 0% for the control. However, this increase in percent supernumerary ribs was not considered toxicologically or biologically significant (Chernoff and Rogers, Journal of Toxicology and Environmental Health; Part B, 7:437-449, 2004, and Chernoff *et al.*, Fundamental and Applied Toxicology 17, 448-453, 1991). Absolute and relative liver weights were slightly, but statistically significantly increased only in unmated F2 adult females treated with 15 or 150 mg/kg bw/day with no corresponding histopathological findings. A

NOEL/NOAEL was established at 3000 mg/kg bw/day for dried mycelial cake (equivalent to 150 mg/kg bw/day of avilamycin activity) in this study, the highest dose tested.

e. Genetic Toxicity Studies

(1) Bacterial Reverse Mutation Assay (Ames Test)

(a) Study Title: "*Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay with Crystalline Avilamycin"

(b) Study Number: 6180-742

(c) Report Number: 6180-742

(d) Report Date: November 3, 2004

(e) Study Director: Michael S. Mecch, M.S.

(f) Performing Laboratory (in-life): Covance Laboratories Inc., Vienna, VA, USA

(g) Experimental Design: The study was conducted based on VICH GL 23. Crystalline avilamycin was evaluated for its ability to induce reverse mutations in four strains of *S. typhimurium* (TA 1535, TA 1537, TA 98, TA 100) and one strain of *E. coli* (WP2uvrA), in the presence or absence of an exogenous mammalian activation system (S9). The procedure used was based on the direct plate method. The test substance was dissolved in dimethylsulfoxide (DMSO). Positive control substances used in the assay included Benzo[α]pyrene, sodium azide, ICR-191, 4-nitoquinoline-N-oxide, 2-nitrofluorene, and 2-aminoanthracene.

(h) Results and Conclusion: Based on the results of the initial mutagenicity assay, doses were selected for the confirmatory assay. With all *S. typhimurium* tester strains, except for TA98, the doses tested in the presence and absence of S9 mix were 0.0100, 0.0333, 0.100, 0.333, 1.00, 3.33, 10.0, and 33.3 µg per plate; the doses tested with the tester strain TA98 in the presence and absence of S9 mix were 0.0100, 0.0333, 0.100, 0.333, 1.00, 3.33, 10.0, 33.3, and 100 µg per plate; the doses tested with the *E. coli* tester strain in the presence of S9 mix were 0.100, 0.333, 1.00, 3.33, 10.0, 33.3, and 100 µg per plate; the doses tested with the *E. coli* tester strain in the absence of S9 mix were 0.0333, 0.100, 0.333, 1.00, 3.33, 10.0, and 33.3 µg per plate.

In the confirmatory mutagenicity assay (except for the mean positive control value for the tester strain TA100 in the presence of S9 mix), no positive increases in the mean number of revertants per plate were observed with any of the tester strains in either the presence or absence of S9 mix. Since the mean positive control value for the tester strain TA100 in the presence of S9 mix did not exhibit at least a 3-fold increase over the mean vehicle control value, the test article was re-tested with TA100 in the presence of

S9 mix; no positive increases in the mean number of revertants per plate were observed.

Under the conditions of this study, crystalline avilamycin did not cause a positive increase in the mean number of revertants per plate with any of the tester strains, either in the presence or absence of the metabolic activation system.

(2) *In Vitro* Mammalian Cell Gene Mutation Test

(a) Study Title: "Mutagenicity Test on Crystalline Avilamycin in the L5178Y $TK^{+/-}$ Mouse Lymphoma Forward Mutation Assay"

(b) Study Number: 6180-721

(c) Report Number: 6180-721

(d) Report Date: October 8, 2004

(e) Study Director: Maria A. Cifone, Ph.D.

(f) Performing Laboratory (in-life): Covance Laboratories Inc., Vienna, VA, USA

(g) Experimental Design: This study followed VICH GL 23 and was conducted to evaluate the ability of crystalline avilamycin to induce forward mutations at the thymidine kinase ($TK^{+/-}$) locus in mouse lymphoma L5178Y cells as indicated by colony growth in the presence of 5-trifluorothymidine (TFT). The vehicle for the test article was DMSO. Rat liver S9 homogenate (prepared from Aroclor 1254-induced male Fisher) was used in the metabolic activation. The positive control substances were ethyl methanesulphonate and methyl methanesulphonate in the absence of S9 mix, and 3-methylcholanthrene in the presence of S9 mix.

(h) Results and Conclusion:

Non-activation Mutation Assay with a 4-Hour Treatment Period: Thirteen treatment levels, at 75.0, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900, and 1000 µg/mL, were initially included. Eight treatments from 100 to 600 µg/mL were cloned. A range of cytotoxicity was induced [85.7% (at 75 µg/mL) to 17.0% (at 1000 µg/mL) relative growths] in the remaining six treatments. None of the analyzed treatments induced a mutant frequency that exceeded the minimum criteria of 142.2×10^{-6} for a positive response.

Non-activation Mutation Assay with a 24-Hour Treatment Period: Thirteen treatment levels, at 2.50, 5.00, 10.0, 15.0, 20.0, 30.0, 40.0, 45.0, 50.0, 55.0, 60.0, 80.0, and 100 µg/mL, were initially included. Eight treatments from 10.0 to 60.0 µg/mL were chosen for mutant analysis, with a range of cytotoxicity observed (93.8% to 29.5% relative growths). None of the analyzed treatments induced a mutant frequency that exceeded the minimum criterion of 129.2×10^{-6} for a positive response.

Activation Mutation Assay with a 4-Hour Treatment Period: Thirteen treatment levels, at 40.0, 50.0, 75.0, 100, 150, 200, 250, 300, 325, 350, 375, 400, and 500 µg/mL, were initially included. Eight treatments from 40.0 to 300 µg/mL were chosen for mutant analysis, and a range of cytotoxicity was observed (101.7% to 12.9% relative growths). None of the analyzed treatments induced a mutant frequency that exceeded the minimum criteria of 108.1×10^{-6} for a positive response.

The average cloning efficiencies for the vehicle controls were 124.8% and 102.8% without activation and 97.2% with S9 metabolic activation, demonstrating acceptable cloning conditions for the assays. The positive control cultures, MMS (non activation) and MCA (activation), induced large increases in mutant frequency that were greatly in excess of the minimum criteria. Mutant colonies from all the cultures showed the expected bimodal distribution, and mutant colonies from MMS and MCA treated cultures showed both small and large colonies.

The test article, crystalline avilamycin was negative for its ability to induce forward mutations at the TK locus in L5178Y mouse lymphoma cells with and without metabolic activation.

(3) *In Vivo* Mammalian Chromosome Aberrations Test

(a) Study Title: "Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells with Crystalline Avilamycin"

(b) Study Number: 6180-738

(c) Report Number: 6180-738

(d) Report Date: November 1, 2004

(e) Study Director: Hemalatha Murli, Ph.D.

(f) Performing Laboratory (in-life): Covance Laboratories Inc., Vienna, VA, USA

(g) Experimental Design: This study followed VICH GL 23 and was conducted to evaluate the ability of crystalline avilamycin to cause structural chromosomal aberrations in Chinese hamster ovary (CHO) cells with and without an exogenous metabolic activation system. DMSO was used as the vehicle control. The metabolic activation system (S9 mix) consisted of liver homogenate (S9) from Aroclor-1254 induced male Sprague-Dawley rat liver and the necessary cofactors. The positive control agents used in the assays were mitomycin C for the non-activation series and cyclophosphamide in the metabolic activation series. A total of 100 metaphase cells in each of the duplicate cultures were evaluated for signs of chromosomal aberration.

(h) Results and Conclusion: In the assay without metabolic activation with a 3-hour treatment, a precipitate was observed after dosing at 450, 525, and 600 µg/mL, and prior to wash and harvest at 375,

450, 525, and 600 µg/mL. Reductions of 2%, 1%, 2%, 14%, and 31% were observed in the mitotic index of the cultures treated with 300, 375, 450, 525, and 600 µg/mL, respectively, as compared with the vehicle control cultures. In the assay without metabolic activation with a continuous 20-hour treatment, a precipitate was observed prior to harvest at 175, 200, 225, 275, 325, and 375 µg/mL. Reductions of 3%, 3%, 2%, 1%, 0%, 19%, and 26% were observed in the mitotic indices of the cultures treated with 150, 175, 200, 225, 275, 325, and 375 µg/mL, respectively, as compared with the vehicle control cultures. No significant increase in chromosomal aberrations, polyploidy, or endoreduplication was observed at the concentrations analyzed either with the 3-hour treatment (225, 300, and 375 µg/mL) or continuous treatment (125, 150, and 175 µg/mL).

In the assay with metabolic activation for a 3-hour treatment, a precipitate was observed after dosing at 450, 525, and 600 µg/mL, prior to wash at 375, 450, 525, and 600 µg/mL, and prior to harvest at 375, 525, and 600 µg/mL. Reductions of 0%, 2%, 2%, and 8% were observed in the mitotic index of the cultures treated with 300, 375, 525, and 600 µg/mL, respectively, as compared with the vehicle control cultures. No significant increase in chromosomal aberrations, polyploidy, or endoreduplication was observed at the concentrations analyzed (225, 300, and 375 µg/mL).

Crystalline avilamycin was considered negative for inducing structural chromosomal aberrations in CHO cells with and without metabolic activation.

(4) *In Vivo* Mammalian Micronucleus Test with Crystalline Avilamycin

(a) Study Title: "The Effect of Granular Avilamycin Given Orally by Gavage for 2 Consecutive Days on the Induction of Micronuclei in Bone Marrow of ICR Mice"

(b) Study Number: M00052

(c) Report Number: M00052

(d) Report Date or Study Dates: October, 2004

(e) Study Director: J.B. Phelps, B.S.

(f) Performing Laboratory (in-life): Eli Lilly and Company, Greenfield, IN, USA

(g) Experimental Design: This study was based on VICH GL 23 and was conducted to investigate the potential of granular avilamycin to induce micronuclei (MN) *in vivo* in bone marrow of male and female ICR mice. Test article suspensions were prepared and stored within established stability parameters at avilamycin concentrations of 0, 25, 50, and 100 mg/mL in purified water. Granular avilamycin was administered orally by gavage to mice (5/sex/group) on days 0 and 1 at doses of 0 (vehicle control), 500, 1000, or 2000 mg/kg bw. Cyclophosphamide, served as the positive control,

was administered by the same route on day 1 at a dose of 50 mg/kg bw. Approximately 24 hours after administration of the second test article treatment, all animals were humanely sacrificed and bone marrow samples were collected from femurs and streaked on slides. Two slides were made from each animal with one slide prepared from each femur. One bone marrow slide from each animal was fixed and stained. Slides were evaluated using the High Capacity Slide Analysis System (HCSA). When possible, at least 2000 anucleate polychromatic erythrocytes (PCE) were counted for each animal. The numbers of PCE with and without MN as well as the number of normochromatic erythrocytes (NCE) were recorded by the system. Bone marrow toxicity was evaluated by dividing the total number of PCE by the total number of NCE to determine the PCE/NCE ratio.

(h) Results and Conclusion: There were no deaths and all animals appeared normal throughout the study. The mean incidence of MPCE/1000 PCE ranged from 0.4 to 0.8 for treated males and from 0.6 to 0.7 for treated females. The mean incidence of MPCE/1000 PCE for the vehicle-control group was 0.8 for males and 0.9 for females. Animals treated with cyclophosphamide, the positive control, had mean MPCE/1000 PCE incidences of 7.8 for males and 5.3 for females, indicating that the test system was sensitive for the detection of clastogenic agents. Poisson dispersion tests of the micronucleus counts in control males and females were not statistically significant. Therefore, a test for a positive trend in Poisson data was performed on the MPCE counts for each sex and for both sexes combined. There were no increases in micronucleus frequencies for males, females, or both sexes combined. Granular avilamycin was not clastogenic and did not interact with the mitotic spindle.

(5) *In Vivo* Mammalian Micronucleus Test with Crystalline Avilamycin

(a) Study Title: "The Effect of Crystalline Avilamycin Given Orally by Gavage for 2 Consecutive Days on the Induction of Micronuclei in Bone Marrow of ICR Mice"

(b) Study Number: M00054

(c) Report Number: M00054

(d) Report Date: October, 2004

(e) Study Director: J.B. Phelps, B.S.

(f) Performing Laboratory (in-life): Eli Lilly and Company, Greenfield, IN, USA

(g) Experimental Design: This study was based on VICH GL 23 and was conducted to investigate the potential of crystalline avilamycin to induce MN *in vivo*, in bone marrow of male and female ICR mice. Test article suspensions were prepared and stored within established stability parameters at avilamycin concentrations of 0,

25, 50, and 100 mg/mL in peanut oil. Crystalline avilamycin was administered orally by gavage to mice (5/sex/group) on days 0 and 1 at doses of 0 (vehicle control), 500, 1000, or 2000 mg/kg bw. Cyclophosphamide, served as the positive control, was administered by the same route on day 1 at a dose of 50 mg/kg bw. Approximately 24 hours after administration of the second test article treatment, all animals were humanely sacrificed and bone marrow samples were collected from femurs and streaked on slides. The formation of micronucleus and bone marrow toxicity were evaluated in the same manner as in Study M000052 (evaluation of granular avilamycin).

(h) Results and Conclusion: There were no deaths and all animals appeared normal throughout the test. The mean incidence of MPCE/1000 PCE ranged from 0.8 to 0.9 for treated males and from 0.7 to 0.8 for treated females. The mean incidence of MPCE/1000 PCE for the vehicle control group was 0.9 for males and females. Animals treated with cyclophosphamide, the positive control, had mean MPCE/1000 PCE incidences of 4.8 for males and 6.3 for females, indicating that the test system was sensitive for the detection of clastogenic agents. Because of cyclophosphamide toxicity, only 3 animals of each sex were evaluated in the positive-control group. Poisson dispersion tests of the micronucleus counts in control males and females were not statistically significant. Therefore, a test for a positive trend in Poisson data was performed on the MPCE counts for each sex and for both sexes combined. There were no increases in micronucleus frequencies for males, females, or both sexes combined. Crystalline avilamycin was not clastogenic and did not interact with the mitotic spindle.

(6) Summary of genotoxicity studies

Table 6. Summary of genotoxicity studies

Study Type	Study Number	Results
Bacterial Reverse Mutation Assay (Ames Test)	6180-742	Negative
In vitro Mammalian Cell Gene Mutation Test	6180-721	Negative
In Vitro Mammalian Chromosome Aberrations Test	6180-738	Negative
In vivo Mammalian Micronucleus Test (with granular avilamycin)	M00052	Negative
In vivo Mammalian Micronucleus Test (with crystalline avilamycin)	M00054	Negative

These study data indicated that avilamycin does not have the potential to cause genetic toxicity in the test systems described above.

f. Oral Carcinogenicity Study in Mice

(1) Study Title: "CGA 59327 (Avilamycin) Potential Tumorigenic Effects in Prolonged Dietary Administration to Mice"

(2) Study Number: 78-9007

(3) Report Number: CBG/186-G/80641

(4) Report Date: August 27, 1982; re-issued June 27, 1983

(5) Study Authors: Brian Hunter, Caroline Graham, Ralph Heywood, David E. Prentice, William A. Gibson, and David Lewis

(6) Performing Laboratory (in-life): Huntingdon Research Center Plc, Huntingdon, Cambridgeshire, England

(7) Experimental Design: The purpose of this study was to evaluate the chronic and tumorigenic effects of avilamycin (CGA-59327) on CD-1 mice after dietary administration for 104 weeks. In this study, mice (60/sex/group) were fed a diet containing mycelial cake (7% avilamycin activity) at 30, 300, or 3000 ppm (equivalent to 3.2, 30.5, and 308 mg/kg bw/day for males and 3.1, 30.2, and 306 mg/kg bw/day for females), or a diet containing pure avilamycin (100% avilamycin activity) at 3000 ppm (equivalent to 310 mg/kg bw/day for males and 324 mg/kg bw/day for females) for 104 weeks. Control animals received untreated diet. Animals were observed for clinical and behavioral signs, palpable masses, mortalities, food consumption, and body weight changes. At study termination, gross pathology was performed, organ weights were obtained, and samples of various tissues from all animals were preserved for microscopical examination.

(8) Results and Conclusion: Body weight gain was reduced in males and females in the 3000 ppm (100% avilamycin activity) dose group during the first 26 weeks, and subsequent body weight gain of these and other dose groups were similar to controls. Food intake was statistically significantly higher in 3000 ppm (both 7% and 100% avilamycin activity) groups during the first 52 weeks of the study; this change was not considered to be of toxicological significance. There was a minimal reduction in efficiency of food utilization in males and females in the 3000 ppm (100% avilamycin activity) group for the first 24 weeks of treatment, but values in the 30, 300, and 3000 ppm (7% avilamycin activity) groups were similar to controls. No significant treatment related effects were noted in any of the other measured parameters.

There was an increase in the incidence of reticulum cell sarcomas but a decrease in the incidence of lymphosarcomas in females in the 3000 ppm (100% avilamycin activity) group. The incidence of total lymphoreticular tumors in this group was elevated in relation to controls (27 vs. 23). However, there were no increase in incidences of reticulum cell sarcomas or total lymphoreticular tumors (lymphosarcoma, reticulum cell sarcoma, lymphorecticular neoplasm) in either sex in 30, 300, 3000 ppm groups (7% avilamycin activity).

These findings were considered not to be toxicological and biological significant. No significant treatment-related effects on the incidence of any other tumor types were found.

Avilamycin was considered not carcinogenic in mice under the conditions of this study. A NOEL/NOAEL was established at 3000 ppm for dried mycelial cake (equivalent to 306 or 308 mg/kg bw/day of avilamycin activity for males and females, respectively), the highest dose tested in this study.

g. Oral Chronic and Carcinogenicity Study in Rats Following *In Utero* Exposure

(1) Study Title: "CGA 59327 (Avilamycin) Long-Term Feeding Study in Rats Following *in Utero* Exposure"

(2) Report Number: CBG 187/80979

(3) Report Date: August 27, 1982; re-issued June 27, 1983

(4) Study Authors: Brian Hunter, Leigh Berryman, Ralph Heywood, Alan E. Street, David E. Prentice, William Gibson, Susan Harling, David Abbott, Chirukanoath Gopinath

(5) Performing Laboratory (in-life): Huntingdon Research Center Plc, Huntingdon, Cambridgeshire, England

(6) Experimental Design: The purpose of this study was to evaluate the toxicological and tumorigenic effects of avilamycin (CGA-59327) on Sprague-Dawley rats following dietary administration for 104 weeks. The study consisted of a premating phase of one-week duration, a reproductive phase of approximately 7-week duration, and a main phase of 104 weeks on treatment. Rats were fed a diet containing mycelium cake (7% avilamycin activity) at 0, 30, 300, or 3000 ppm (equivalent to 0, 1, 11, and 111 mg/kg bw/day in males and 0, 1, 12, and 128 mg/kg bw/day in females), or a diet containing pure avilamycin (100% avilamycin activity) at 3000 ppm (equivalent to 108 mg/kg bw/day for males and 127 mg/kg bw/day for females).

During premating and reproductive phases (50/sex/group), parental parameters (mortality, food consumption, body weight gain, efficiency of food utilization, pregnancy rates) and litter parameters (litter size, litter and mean pup weights, and pup mortality) were evaluated.

The main carcinogenicity/toxicity study phase consisted of main groups (50 rats/sex/group) and satellite groups (30 rats/sex). Following 104-week treatment, all surviving rats in the main group received untreated diet, and sacrificed after 108 weeks (females) or 12 weeks (males), when the group reached a 20% survival. The satellite groups were selected for blood samples (weeks 12, 25, 51, 77, and 103) and urine samples (13, 27, 52, 78, and 104), and were sacrificed at 52 or 104 weeks. The following parameters were assessed: clinical signs, food and water consumptions, body weight, food efficiency, ophthalmoscopy, urinalysis, hematology, clinical chemistry, gross pathology, organ weights, and microscopic examination for various tissue samples.

(7) Results and Conclusion: Group mean food intake in females was statistically significantly higher in the 3000 ppm (7% avilamycin activity) group, and was also higher in males, but not statistically significant. Overall body weight gain was reduced in females in the 3000 ppm (100% avilamycin activity) group. Thrombotest times were decreased at weeks 13, 26, 52, and 78 in males at 3000 ppm (~7% avilamycin activity) and males at 3000 ppm (100% avilamycin activity). None of the above changes were considered toxicologically or biologically significant. No treatment-related effects on the incidence of any tumor type, reproductive parameters, litter parameters (litter size, litter and mean pup weights, and pup mortality) or any other measured parameters were found.

Avilamycin was considered not carcinogenic in rats under the conditions of the study. A NOEL/NOAEL was established at 3000 ppm (5% avilamycin activity) for mycelial cake (equivalent to 128 mg/kg bw/day for males and 111 mg/kg bw/day for females), the highest dose tested.

h. Other Non-Pivotal Studies

In a 28-day oral toxicity study in rats (Study No. Siss 6265), 80 Tif rats (10/sex/group) were fed a diet containing the test material (10% avilamycin) at 0, 30, 300, or 3000 ppm for 28 days. Animals were observed daily for clinical signs, body weight changes and food consumptions, and eye examinations were performed weekly. Blood and urine samples were collected at the end of the study on 40 randomized rats from each group for hematological and clinical chemistry analysis. An agarose gel electrophoresis was performed on protein. No treatment related effects were noted at any of the doses tested. A NOEL/NOAEL was not established for this study.

In another 28-day study in rats (Study No. Siss 6354), 40 Tif rats (10/sex/group) were fed a diet containing avilamycin in the mycelial form at 0 and 30,000 ppm for 28 days. The purity of the test material was not provided in the report. Animals were observed daily for clinical signs, body weight changes and food consumption, and eye examinations were performed weekly. Blood and urine samples were collected at the end of the study from each group for hematological and clinical chemistry analysis. An agarose gel electrophoresis was performed on protein. No treatment related effects were noted at any of the doses tested. A NOEL/NOAEL was not established for this study.

In a 28-day study in mice (Study No. Siss 6265), 80 Tif:MAGf (SPF) mice (10/sex/group) at approximately 4 weeks of age were fed a diet containing the test material (10% avilamycin) at 0, 30, 300, and 3000 ppm (equivalent to 4.69, 45.68, and 415.17 mg/kg bw/day for males, and 5.21, 50.92, and 618.49 mg/kg bw/day for females) for 28 days. Animals were observed daily for clinical signs, body weight changes and food consumption, and eye examinations were performed. A slight increase in body weight and food consumption was noted in males in the 3000 ppm group. No treatment related effects were noted at any of the doses tested. A NOEL/NOAEL was not established in this study.

In another 28-day study in mice (Study No. Siss 6354), 40 Tif:MAGf (SPF) mice (10/sex/group) at approximately 4 weeks of age were fed avilamycin-mycel in the diet at 0 and 30,000 ppm (equivalent to 6117 mg/kg bw/day for males and 6108 mg/kg bw/day for females) for 28 days. Animals were observed daily for clinical signs, body weight changes and food consumption, and eye examinations were performed weekly. The purity of the test material was not provided in the report. A slight increase in food consumption was noted in males in the 30,000 ppm group. No treatment-related effects were noted at any of the doses tested. A NOEL/NOAEL was not established for this study.

In other acute and pilot oral toxicity studies in rats or mice, there were no significant treatment-related effects observed. A reproductive safety study of feeding avilamycin to gilts during the growing-finishing phase also supported that avilamycin is not an overt reproductive toxicant in mammalian species.

2. Determination of Toxicological No-Observed-Effect Level (NOEL)/No-Observed-Adverse-Effect Level (NOAEL) for Chronic Exposure

Studies for total residues of avilamycin considered for determination of the toxicological NOEL/NOAEL for chronic exposure are summarized in Table 2.

Table 7. No-Observed-Effect Levels/No-Observed-Adverse-Effect Levels in toxicology studies for avilamycin

Study Type	Study Number	NOEL/NOAEL (mg/kg bw/day)
Chronic Oral Toxicity Study in Dogs	D03782	178
Developmental Toxicity Study in Rats	R00228	528
Developmental Toxicity Study in Rabbits	B03482	356
Three-generation Reproduction Study in Rats	CBG/188/80780	150
Oral Carcinogenicity Study in Mice	78-9007	306
Oral Carcinogenicity Study in Rats	CBG187/80979	111

The NOEL/NOAEL of 111 mg/kg bw/day from the 104-week oral carcinogenicity study in rats (Study Number CBG187/80979) was selected for determination of the toxicological ADI for chronic exposure of total residues of avilamycin to human consumers.

3. Determination of Toxicological ADI

Based on the available toxicology studies, the 104-week oral carcinogenicity study in rats was determined to be the most appropriate study to determine the toxicological acceptable daily intake (ADI) for total residues of avilamycin. A safety factor of 100 was applied because the NOEL was from a 2-year carcinogenicity study and no significant biological or toxicological effects were

shown in any of the endpoints examined. The toxicological ADI is calculated using the following formula.

$$ADI = \frac{NOEL/NOAEL}{Safety\ \ Factor} = \frac{111\ \ mg/kg\ \ \ bw/day}{100}$$

$$= 1.1\ \ 1\ mg/kg\ \ \ bw/day\ \ \ = 1110\ \ microgram/\ \ \ \ kg\ \ bw/day$$

The toxicological ADI for avilamycin is 1.11 mg/kg bw/day or 1110 µg/kg bw/day.

D. Assignment of the Final ADI

Because avilamycin has limited effects on human intestinal flora and a microbiological ADI was not needed, we assign the toxicological ADI (1.11 mg/kg bw/day or 1110 µg/kg bw/day) as the final ADI for total residues of avilamycin.

E. Safe Concentrations for Total Residues in Edible Tissues

1. The calculation of the tissue safe concentrations is based on the General Principles for Evaluating the Safety of Compounds used in Food-Producing Animals (FDA/CVM, revised July 2006). The safe concentration of total residues of avilamycin (ppm) in each edible tissue of swine is calculated using the following formula:

$$Safe\ \ Concentrat\ \ \ ion = \frac{Acceptable\ \ \ Daily\ \ Intake\ \ (ADI)\ \ x\ Human\ \ \ Body\ \ Weight}{Food\ \ Consumptio\ \ \ n\ Value}$$

The average human body weight is approximated at 60 kg. The daily food consumption values of each edible tissue of swine are approximated as 300 g for muscle, 100 g for liver, 50 g for kidney, and 50 g for fat.

2. Safe Concentrations of Total Residues of Avilamycin in Edible Tissues of Swine Using the Food Consumption Values

Table 8. Summary Table of Safe Concentrations for Total Residues

Edible Tissue	Amount Consumed Per Day	Safe Concentration
Muscle	300 g	220 ppm
Liver	100 g	660 ppm
Kidney	50 g	1320 ppm
Fat	50 g	1320 ppm

F. Residue Chemistry

1. Summary of Residue Chemistry Studies

a. Total Residue and Metabolism Studies

Title: "A Steady-State Tissue Residue Study in Swine Dosed with Uniformly Labeled ^{14}C Avilamycin." Study ABC-0360.

This study was conducted with adherence to appropriate FDA and OECD Good Laboratory Practices (GLPs).

Study Director: J.D. Magnussen, M.S.

Study Dates: September 15, 1986-March 12, 1987

Study Facility: Lilly Research Laboratories, Greenfield, IN

Seven crossbred swine (five barrows and two gilts), weighing between 37.5 -42 kg, were fed at 12-hour intervals for either 10 or 14 days with a ration containing a nominal concentration of 60 mg of uniformly-labeled ^{14}C avilamycin/kg of feed (equivalent to 60 mg activity/kg and to 3.6-4.8 mg/kg bw/day). Six hours after the final feeding on days 10 and 14, animals were slaughtered and samples of liver, kidney, muscle and fat were collected. Urine and feces were collected at 24-hour intervals from one animal in the 10-day dose group. Total radioactivity in tissue, urine and feces was measured by combustion and liquid scintillation counting (LSC). The metabolic profile was determined using silica gel column chromatography. Radioactivity in fat was characterized using HPLC.

Table 9. Total Radiolabel Residues (TRR) measured as mg/kg equivalents avilamycin in swine tissues

Dosing Interval (Days)	Animal Number	TRR Muscle	TRR Liver	TRR Kidney	TRR Fat
10	960	0.074	0.534	0.397	0.183
	961	0.071	0.422	0.232	0.234
	957	0.135	0.705	0.335	0.366
	Mean ± Std Dev	0.093 ± 0.04	0.554 ± 0.14	0.321 ± 0.08	0.261 ± 0.09
14	954	0.138	0.77	0.35	0.562
	955	0.102	0.477	0.331	0.513
	959	0.164	0.73	0.34	0.579
	Mean ± Std Dev	0.135 ± 0.03	0.659 ± 0.16	0.34 ± 0.01	0.551 ± 0.03

All tissue concentrations were less than 0.8 ppm. There was no statistical difference between 10 or 14 days for muscle, liver, or kidney total radioactive residues, showing that steady-state concentrations were attained in these tissues. Fat radioactive residues were significantly different ($p < 0.05$) at 10 and 14 days, however, residues are so much lower than the safe concentrations that this is not a concern.

Table 10. Radioactivity excreted and measured in a barrow dosed orally for 10 days with 60 ppm of ^{14}C avilamycin. Values are expressed in % of applied dose.

Collection Period (day)	Urine	Feces
1	0.03	0.05
2	0.04	0.75
3	0.06	0.71
4	0.06	0.76
5	0.06	0.68
6	0.06	0.76
7	0.08	0.85
8	0.06	0.76
9	0.07	0.72
10	0.07	0.65
Mean ± Std Dev	0.06 ± 0.01	0.67 ± 0.23

The majority of the radioactivity was recovered in the feces (67%). Approximately 6% of the radioactivity was recovered in the urine.

Table 11. Fractionation of radioactivity as percent of total in livers of six swine

Treatment Group	Animal Number	Acetone Extract	Methanol Extract	Acetone/ Water	Pellet
10-day	961	34	25	7	34
	960	32	24	7	37
	957	34	26	6	33
14-day	954	32	25	8	34
	955	33	26	6	35
	959	29	28	6	37

Non-extractable liver residues were 33-37% of total liver residues and were not different in the 10- and 14-day treatment groups. Extractable liver radioactivity consisted of several minor metabolites (< 0.1 ppm), none of which were major metabolites. The most abundant metabolite was flambic acid, present at concentrations up to 0.06 ppm. Flambic acid was also present in swine and rat excreta and in rat liver.

b. Comparative Metabolism Study

Title: "Comparative Metabolism of ^{14}C Avilamycin in Swine and Rats Experiment." ABC-0371.

The purpose of this GLP-study was to compare the metabolic profile of ^{14}C avilamycin in swine, the target animal, with rats, the toxicological experimental animal.

This study was conducted with adherence to FDA and OECD Good Laboratory Practices (GLPs).

Study Dates: October 29, 1986 - April 2, 1987

Study Director: A.L. Donoho, Ph.D.

Study Facility: Lilly Research Laboratories, Greenfield, IN

Six Sprague-Dawley rats (three males and three females), weighing between 183-278 g, were fed a ration containing 550 ppm of uniformly labeled ^{14}C avilamycin for 4.5 days. Urine and feces were collected during the dosing period and livers were collected at sacrifice. Urine, feces and liver tissue were collected from seven crossbred swine (five barrows and two gilts), weighing between 37.5 and 42 kg, fed the same lot of avilamycin. The swine excreta and liver tissue were obtained from swine used during Study ABC-0360. Radioactivity was determined by combustion and liquid scintillation counting (LSC). Chromatographic profiles were determined by silica gel column chromatography and thin-layer chromatography (TLC). The metabolite pattern in urine, feces and livers of treated swine was similar to that found in rats.

c. Study to Establish Withdrawal Period

Title: "Non-Clinical Laboratory Study (GLP): Avilamycin Residue Decline Study in Swine." Study No. 50405.

This study was conducted with adherence to FDA Good Laboratory Practices (GLPs).

Study Dates: May 12, 2006-December 22, 2006

Study Director: Larry S. Eichmeier, Ph.D.

In-Life Testing Site: Sinclair Research Center, Inc., Auxvasse, MO

Testing Facility and Analytical Laboratory (DIA Method): ABC Laboratories, Inc., Columbia, MO

Analytical Laboratory (Antimicrobial Assay Method): Covance Laboratories, Inc., Madison, WI

Fourteen crossbred commercial pigs (seven castrated males and seven gilts) weighing 8.7-14.9 kg, were fed *ad libitum* a commercial diet containing avilamycin at 150 ppm for 21 days. Tissue samples from the entire liver, both kidneys, loin muscle, fat (abdominal or perirenal fat), and skin+fat (subcutaneous) were collected at the end of the 21-day exposure period at withdrawal intervals of 0, 6, and 24 hours (n = 4/group; 2 males and 2 females). Avilamycin and/or its metabolites were determined in swine tissues using an HPLC-MS/MS method.

Residues were only detected in liver tissue and declined by more than half in 6 hours. After 24 hours, residues were below or near the limit of quantitation. No residues were detected in muscle or fat/skin at any time. Residues were detected, but not quantifiable, in kidney at 0 and 6 hours withdrawal, and were not detected at 24 hours.

Table 12. Final Mean Concentrations of DIA/Avilamycin (ng/g) in Swine Tissue Samples measured by LC-MS/MS

Withdrawal Time (hour)	Sample ID	Sample Type	Liver	Fat/Skin	Muscle	Kidney
0	1M1	Control	ND	ND	ND	ND
	1F1	Control	ND	ND	ND	ND
	Mean ± Std Dev		ND	ND	ND	ND
0	2M1	Treated	94.6	ND	ND	BQL
	2M2	Treated	103	ND	ND	BQL
	2F1	Treated	138	ND	ND	BQL
	2F2	Treated	75.2	ND	ND	BQL
	Mean ± Std Dev		102.7 ± 26.3	ND	ND	BQL
6	3M1	Treated	41.5	ND	ND	BQL
	3M2	Treated	29.6	ND	ND	BQL
	3F1	Treated	45.9	ND	ND	BQL
	3F2	Treated	49.5	ND	ND	BQL
	Mean ± Std Dev		41.6 ± 8.7	ND	ND	BQL
24	4M1	Treated	BQL	ND	ND	ND
	4M2	Treated	BQL	ND	ND	ND
	4F1	Treated	32	ND	ND	ND
	4F2	Treated	BQL	ND	ND	ND
	Mean ± Std Dev		32	ND	ND	ND

*ND = Not Detected = < Limit of Detection
*BQL = Below Quantifiable Limit

2. Target Tissue and Marker Residue

Neither a target tissue nor marker residue are assigned.

3. Tolerance(s)

A tolerance for avilamycin is not needed.

4. Withdrawal Period

Tissue residue data from Study ABC-0360 and Study No. 50405 support a zero-day withdrawal period for avilamycin.

G. Analytical Method for Residues

1. Description of Analytical Method

Because a tolerance has not been assigned, a validated analytical method is not necessary.

V. USER SAFETY

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to KAVAULT:

Avilamycin may be irritating to the eyes and may cause allergic reactions in those hypersensitive to avilamycin. Avoid inhalation, oral exposure, and direct contact with skin or eyes. Operators mixing and handling KAVAULT should use protective clothing, impervious gloves, goggles, and an approved dust mask. Wash hands thoroughly with soap and water after handling. If accidental eye contact occurs, immediately rinse thoroughly with water and seek medical attention. If wearing contact lenses, rinse the eyes first, then remove contact lenses and continue to rinse the eyes thoroughly and seek medical attention. If accidental skin contact occurs, wash all exposed areas of skin thoroughly with soap and water, and seek medical attention if irritation develops. If accidental inhalation occurs, seek medical attention if breathing difficulty occurs. Not for human consumption. If accidental ingestion occurs, call a physician or poison control center. Do not induce vomiting. Keep out of reach of children. The Safety Data Sheet contains more detailed occupational safety information. To report adverse effects in users, to obtain more information, or to obtain a Safety Data Sheet, call 1-800-428-4441.

VI. AGENCY CONCLUSIONS

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR part 514. The data demonstrate that KAVAULT, when used according to the label, is safe and effective for the reduction in incidence and overall severity of diarrhea in the presence of pathogenic *Escherichia coli* in groups of weaned pigs. Additionally, data demonstrate that residues in food products derived from weaned pigs treated with KAVAULT will not represent a public health concern when the product is used according to the label.

A. Marketing Status

A valid veterinary feed directive (VFD) is required to dispense this drug. Any animal feed bearing or containing this drug will be fed to animals only by or on a lawful veterinary feed directive issued by a licensed veterinarian in the course of their professional practice.

Labeling restricts this drug to use under the professional supervision of a licensed veterinarian. The decision to restrict this drug to use by or upon a lawful VFD issued by a licensed veterinarian was based on the following factors: (a) adequate directions cannot be written to enable lay persons to appropriately and safely use this product and (b) restricting this drug to use by or upon a lawful VFD issued by a licensed veterinarian should help prevent indiscriminate use, which could result in violative tissue residues.

B. Exclusivity

KAVAULT, as approved in our approval letter, qualifies for FIVE years of marketing exclusivity beginning as of the date of our approval letter. This drug qualifies for exclusivity under section 512(c)(2)(F)(i) of the Federal Food, Drug, and Cosmetic Act because this is the first time we are approving this active ingredient in a new animal drug application submitted under section 512(b)(1) of the FD&C Act.

C. Patent Information

For current information on patents, see the Animal Drugs @ FDA database or the Green Book on the FDA CVM internet website.